Alpha-, Beta-, and Gamma-Synuclein: Developmental Localisation and Response to Cellular Insult in Neurons and Oligodendrocytes.

by

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Part of the work presented in this thesis has been published or submitted for publication as follows;

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Quilty, M.C., W-P. Gai, D.L. Pountney, A.K.West, and J.C.Vickers. Localisation of alpha-, beta- and gamma-synuclein during neuronal development and alterations associated with the neuronal response to axonal trauma. *Experimental Neurology*. 182 (2003), 195-207.

Pountney, D.L., Lowe, R., **Quilty M.C**., Vickers, J.C., Voelcker N.H., Gai, W.-P. Annular alpha-synuclein species from purified multiple system atrophy inclusions. *Journal of Neurochemistry* 90 (20) (2004), 502-12.

SUMMARY

Alpha-synuclein is a highly conserved, ubiquitously expressed protein in the vertebrate brain. It was discovered in 1988 and was purported to play a role in song learning, implicating a role in synaptic plasticity. Alpha-synuclein came under increased scrutiny shortly thereafter as the non-amyloid component isolated from plaques from Alzheimer's disease brains. Following this discovery, alpha-synuclein was further characterised as the main component in numerous additional inclusion bodies found in several neurodegenerative disease leading to speculation that alpha-synuclein was involved in disease pathogenesis.

Two decades later little is known about the normal function of alpha-synuclein in neuronal cells, however numerous studies have determined alpha-synuclein is able to form fibrils and aggregates, and that these may provide the neurotoxic mechanism by which alpha-synuclein exerts its effect in disease states. Other studies have demonstrated the formation of fibrillar aggregates of alpha-synuclein is a neuroprotective mechanism. The issues of the cellular role and neurotoxicity of alpha-synuclein remain contentious. This thesis provides evidence indicating a developmentally regulated role for alpha-synuclein in neurons and oligodendrocytes, as well as roles in neuroprotection, plasticity and regeneration. This thesis has demonstrated synuclein isoforms have a distinct pattern of localisation during neuronal development, indicating alpha-, beta- and gamma-synuclein also have distinct functionality within neuronal development, and that these functions may alter as neurons develop.

Alpha-synuclein immunoreactive neuritic abnormalities were shown to be caused by physical insult to the axon, leading to the accumulation of alpha-synuclein protein in neuronal processes, and were associated with neurofilament pathology mirroring that seen in some disease states containing aggregated forms of alpha-synuclein. It was demonstrated alpha-synuclein is involved in regenerative sprouting following axonal injury, further implying a role for alpha-synuclein in aspects of growth cone development and/or maturation as well as neuronal plasticity.

Additionally, synuclein isoforms were shown to have a distinct pattern of localisation during oligodendroglial development, indicating alpha-, beta- and gamma-synuclein also have distinct functionality within oligodendrocytes, and these functions may alter throughout development. In this case exogenous alpha-synuclein was demonstrated to be taken up by oligodendrocytes, and incorporated into cytoplasmic inclusions, along with endogenous alpha-synuclein, implying a mechanism by which alpha-synuclein interacts with oligodendrocytes in disease states leading to the formation of glial cytoplasmic inclusions. Finally, and importantly this thesis demonstrated an increase in alpha-synuclein in response to stressful stimuli is beneficial to neuronal survival as part of a normal neuronal response in a subpopulation of neurons. This finding is somewhat contentious as many of the studies into the role and mechanism of alpha-synuclein in disease states support its neurotoxicity, yet this thesis contradicts this, providing evidence for a neuroprotective affect of alpha-synuclein.

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TABLE OF CONTENTS

ABBREVIATIONS

aa	amino acid
AD	Alzheimer's disease
ALS	amyotrophic lateral sclerosis
ATP	adenosine triphosphate
A53T	missense mutant alpha-synuclein
A30P	missense mutant alpha-synuclein
BAD	Bcl-associated death promoter
CNS	central nervous system
CSF	cerebrospinal fluid
CSP-alpha	cysteine-string protein alpha
DA	dopamine
DAI	diffuse axonal injury
DAT	dopamine transporter
DHR-123	dihyrdrorhodamine – 1,2,3
DIV	days *in vitro*
DLB	dementia with Lewy bodies
DLBD	diffuse Lewy body dementia
DMEM	Dulbecco's Modified Eagle's Medium
EDTA	ethylenediamine tetra acetic acid
ER	endoplasmic reticulum
ERK	extracellular signal regulated kinase
E46K	missense mutant alpha-synuclein
GCI	glial cytoplasmic inclusion
GNI	glial nuclear inclusion
GRK	G protein coupled receptor kinase
HBSS	Hank's Balance Salt Solution
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid
IR	immunoreactivity
kDa	kilo dalton
LB	Lewy body
LBVAD	Lewy body variant of Alzheimer's disease
LN	Lewy neurite
LRRK2	Leucine-rich repeat kinase 2
MAO	mono-amine oxidase

MAP	microtubule-associated protein
MPTP	1-methyl-1-phenyl-1,2,3,6-tetrahydropyridine
mRNA	messenger ribonucleic acid
MSA	multiple systems atrophy
NA	noradrenaline
NAC	non-amyloid component
NACP	non-amyloid component precursor protein
NCI	neuronal cytoplasmic inclusion
NFH	neurofilament heavy chain
NFL	neurofilament light chain
NFM	neurofilament medium chain
NFT	neurofibrillary tangle
NNI	neuronal nuclear inclusion
OE	olfactory epithelium
ORN	olfactory receptor neuron
PARK1	alpha-synuclein gene
PBS	phosphate buffered saline
PD	Parkinson's disease
PFA	paraformaldehyde
PINK1	phosphatase and tensin (PTEN) homologue - induced putative kinase 1
PKC	protein kinase C
PM	plasma membrane
PNP-14	phosphoneuroprotein 14 kDa
RNA	ribonucleic acid
ROS	reactive oxygen species
SEM	standard error of the mean
SN	substantia nigra
SNCA	alpha-synuclein gene
SNpc	Substantia nigra pars compacta
SNpr	Substantia nigra pars reticulata
SOD	superoxide dismutase
TH	Tyrosine hydroxylase
WT	wild type